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Introduction

The EZgene™ 96 MagBind Plasmid Purification Kit combines the power of MagBind technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality plasmid DNA. By using 96-well format, up to 96 samples can be simultaneously processed in less than 60 minutes. This kit provide an average DNA recovery rate 10 to 30% higher than the manual centrifuge method. Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, but 1 ml of overnight culture in LB medium typically produces 8-12 ìg high-copy plasmid DNA. The purified plasmid can be used directly for automated fluorescent DNA sequencing, such as with BigDye sequencing chemistry, as well as for other standard molecular biology techniques including restriction enzyme digestion.

Storage and Stability

All EZgene™ MagBind Plasmid Purification gDNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows:

- RNaseA store at -20°C.
- MagBind Particles MBP store at 4°C.
- All other materials at room temperature (22-25°C).

Kit Contents

Catalog #	M1812-00	M1812-01	M1812-02
Preps	1 x 96	4 x 96	10 x 96
MagBind Particles MBP	2 mL	8 mL	20 mL
Buffer A1	12 mL	50 mL	120 mL
Buffer B1	12 mL	50 mL	120 mL
Buffer M1	12 mL	50 mL	120 mL
Buffer MKB	15 mL	60 mL	150 mL
DNA Wash Buffer	24 mL	4 x 24 mL	10 x 24mL
Elution Buffer	10 mL	40 mL	100 mL
96-Well Lysate Clearance Plate	1	4	10
96- Well 1.6 mL Plate	1	4	10
96-Well Collection Plate	2	8	20
RNase A (20 mg/mL)	90 µL	360 µL	900 µL
User Manual	1	1	1

Before Starting

The kit is designed to be simple, fast, and reliable provided that all steps are followed diligently. Please read the entire user manual and get all necessary supplies and equipments.

Important

- RNase A: **20 mg/mL**. It is stable for more than half a year when stored at room temperature. Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use. Store at 4°C.
- Add **96 mL 96-100% ethanol** to each **DNA Wash Buffer** bottle before use.
- Add **18 mL** (**M1812-00**),**72 mL** (**M1812-01**),**180 mL**(M1812-02) **96-100% ethanol** to each **MagBind Particles MBP** bottle before use.
- Add **15 mL** (**M1812-00**),**60 mL** (**M1812-01**),**150 mL**(M1812-02) **96-100% ethanol** to each **Buffer MKB** bottle before use.
- **Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.**
- Keep the cap tightly closed for Buffer B1 after use.
- **Carry out all centrifugations at room temperature.**
- **Shake or vortex the Mag-Bind solution to fully resuspend the particles.** During use,the particles must be fully suspended to assure proper binding.

Materials provided by user

- Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g (such as Eppendorf 5810 with MTP rotor).
- Water bath set to 65°C and 70°C.
- Absolute ethanol(96%-100%)
- Magnetic separation device for microcentrifuge tubes or microplates.

EZgene™ 96 MagBind Plasmid Purification Kit

1. Inoculate **1-1.5 mL** LB containing appropriate antibiotic in a 96-well 2ml culture plate. Incubate at 37°C for 14-16 hours with shaking.

   Note: Prolonged incubation (> 16 hours) is not recommended since the *E.coli* starts to lyse and the plasmid yields may be reduced.
   Note: Do not grow the culture directly from the glycerol stock.
   Note: This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers need to be scaled up proportionally if over amount of cultures are being processed.

2. Harvest the bacterial culture by centrifugation for 10 min at 3000 x g. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   Note: Residue medium will cause,
   - Poor cell lysis and thus lower DNA yield.
   - Loose pellet after centrifugation in step 6.

3. Add **100 µL Buffer A1** (Add RNase A to **Buffer A1** before use) in each well of the deep well plate and completely resuspend bacterial pellet by vortexing or pipetting .

   Note: Complete resuspension is critical for bacterial lysis and lysate neutralization.

4. Add **100 µL Buffer B1** in each well of the deep well plate, mix gently by inverting the tube 10 times (do not vortex), and incubate at room temperature for 5 minutes.

   Note: Do not incubate for more than 5 minutes.
   Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 50°C to dissolve precipitation before use.

5. Add **100 µL Buffer M1**, mix completely by inverting/shaking the plate until a flocculent white precipitate forms.

   Note: Incubating the lysate in ice for 1 min will improve the yield.
   Note: It is critical to mix the solution well. If the mixture still appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution.

6. Centrifuge the plate contains bacterial lysate at 4000 x g for 15 minutes at room temperature.

   Note: Biomiga's Lysate Clearance Plate can also be used to clear cell lysate without long centrifugation and avoid carryover oprecipitates (Cat# BPD002)

7. Transfer **200µL cleared supernatant** into the 96-Well Collection Plate.

8. Add equal volume of MagBind Particles MBP/ethanol solution (**200µL** /well) and mix throughly by pipetting up and down for 20 times.

   Note: The MagBind Particles will settle together at the bottom of the container after several hours. Please check container before use. If beading has occurred, gently shake or vortex container until particles have been redispersed in solution. (IMPORTANT).

9. Place the plate onto the magnetic separation stand to magnetite the magnetic particles. Remove the supernatant after the magnetic particles have completely migrated to the walls of each well adjacent to the magnets. (Supernatant should be clear when migration is complete.)

10. Carefully aspirate and discard the supernatant from the plate.

11. **Optional:** Remove plate containing the MagBind particles from the magnetic separation device. Add **300 µL Buffer MKB** diluted with **absolutely ethanol** to each sample. Resuspend the MagBind Particles pellet by vortexing.

12. Place the plate on the magnet separation stand. Remove and discard the supernatant after the magnetic particles have completely migrated to the walls adjacent to the magnets.

13. Remove plate containing the MagBind particles from the magnetic separation device. Add **300 µL DNA Wash Buffer** diluted with **absolutely ethanol** to each sample.

14. Resuspend the MagBind Particles pellet by vortexing. Incubate 1 minute at room temperature.

Note: Complete resuspension of the MagBind Particles pellet by pipetting up and down or vortexing is critical to obtain good results.

15. Repeat 12-14 Step again.

16. **Optional:** Add **200 µL absolute ethanol** and resuspend the Mag-Bind particles by vortexing or pipetting up and down. Magnetize the Mag-Bind particles then remove the supernatant.

17. Leave the plate on the magnetic separation device. Dry the plate at room temperature or 37°C for 10 minutes.

18. Resuspend the MagBind Particles pellet with **50-100µL Elution Buffer** or **Sterile Water** (7<PH<8) by pipetting up and down for 10 times.

    Note: We recommend using Elution Buffer to eluent as magnetic particles migrate much fatster when using Elution Buffer.

19. Place the plate onto the magnetic separation stand to pellet the Mag-Binds particles.

20. Transfer the supernatant containing the purified plasmid into 96-Well Collection Plate.

Trouble Shooting Guide

Problem	Cause	Possible Solution
Low DNA yield	Poor cell lysis	Do not use more than 2 ml with high copy plasmids. Cells may not be dispersed adequately prior to addition of Buffer B1. Vortex cell suspension to completely disperse. Increase incubation time with Buffer B1 to obtain a clear lysate. Buffer B1 if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1µg DNA from a 1 ml overnight culture.
	Bacterial culture overgrown or not fresh.	Do not incubate cultures for more than 16 hr at 37 ° C. Storage of cultures for extended periods oprior to plasmid isolation is detrimental.
	Lost MagBind Particles during operation	Careful remove the supernatant when aspirating the supernatant during process.
No DNA eluted	Forget to add ethanol to the DNA wash buffer	Prepare the DNA wash buffer as instructed.
Chromosomal DNA contamination	Over mixing after adding Buffer B1	Do not vortexing or vigorously mixing after buffer B1 is added.
DNA flow out of agarose gel when loading	Trace ethanol contamination	Recentrifuge or vacuum again the plate as instructed if necessary.
RNA visible on agarose gel	Forget adding the RNase A to Buffer A1	Add RNase A to Buffer A1.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.
This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (858) 603-3219 or visit our website at www.biomiga.com.